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GRANT NO: DAMD17-93-J-3010

TITLE: A NEW CLASS OF MEMBRANE ACTIVE DRUGS FOR THE TREATMENT OF BREAST CANCER CELLS TRANSPLANTED INTO ATHYMIC MICE

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*Original contains color plates: All DTIC reproductions will be in black and white.

REPORT DATE: December 6, 1993

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research, Development, Acquisition and Logistics Command (Provisional), ATTN: SGRD-RMI-S
Fort Detrick, Frederick, MD 21702-5012

DISTRIBUTION STATEMENT: Approved for public release; distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	6 December 1993	Annual (12/1/92 - 11/30/93)

4. TITLE AND SUBTITLE
A New Class of Membrane Active Drugs for the Treatment of Breast Cancer Cells Transplanted into Athymic Mice

5. FUNDING NUMBERS
Grant No.
DAMD17-93-J-3010

6. AUTHOR(S)
Kirpal S. Gulliya, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Baylor Research Institute
3812 Elm Street
P.O. Box 710699
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research, Development, Acquisition and Logistics Command (Provisional), ATTN: SGRD-RMI-S
Fort Detrick, Frederick, MD 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

This study was performed to determine the efficacy of novel photoproducts in preactivated merocyanine 540 (pMC540) against human breast cancer cells (MCF-7) transplanted into athymic mice. Treatment of serially propagated solid breast xenografts with pMC540 (250 mg/kg) caused a significant (43%) suppression ($P = .004$; $P = .0882$; $P = .0903$) in tumor area, weight, and volume, respectively. Treatment with tamoxifen alone was ineffective. However, a combination of tamoxifen and pMC540 (100 mg/kg) caused a 67% suppression of breast tumor growth. Treatment of implanted tumor with 20 doses (75 mg/kg) of merodantoin, a chemically synthesized photoproduct originally isolated from pMC540, suppressed the tumor growth by 98%. These results show for the first time that photoproducts in pMC540 by themselves and in combination with tamoxifen are effective in suppressing the *in vivo* growth of transplanted human breast tumor.

14. SUBJECT TERMS
Breast cancer, Growth suppression, Merodantoin, pMC540, Tamoxifen, Lab Animals, Mice, RAD VI

15. NUMBER OF PAGES

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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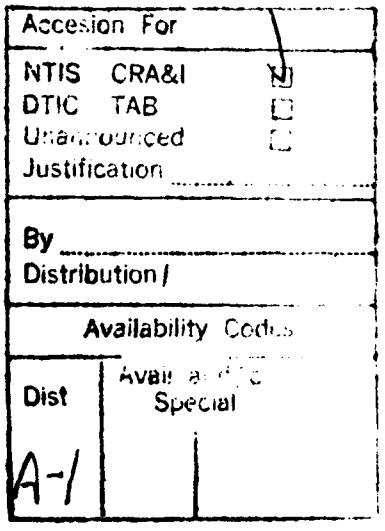

Joel S Gulliya 12/13/93

PI - Signature Date

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Background:

Breast cancer affects one in nine American women (1). Breast cancer as a disease or its treatment has evoked stronger emotions than perhaps any other disease. The reasons for this can be found both in our culture in general and in medicine in particular. Depending upon the culture and context, the breast is the symbol of motherhood, nourishment, security and represents beauty and femininity.

Breast cancer has a long natural history which may provide a misleading estimate of the long term outcome. An important aspect of current breast cancer management is the early detection of tumors by mammographic screening. This strategy has provided important information regarding the natural history of the disease. However, even among women in whom earliest detectable tumor is effectively treated locally, a significant number of these patients develop clinically evident distant disease, presumably micrometastases which occur at the time of initial treatment. Approximately 25% of the patients with Stage I tumors (< 2 cm without axillary lymph node metastasis) and 75% of the patients with Stage II tumors (> 2 cm), develop distant metastasis despite local and regional therapy (2).

Two major forms of therapy that have been considered thus far are hormonal manipulation that can have profound effect on certain estrogen positive advanced breast cancers and chemotherapy with or without radiation therapy. More recently new therapeutic modalities such as taxol (a complex natural product derived from the bark of the Pacific yew tree), immunomodulators, antigrowth factors and tumor infiltrating lymphocytes are in different stages of development. Most chemotherapeutic agents exert their cytotoxic effects in a dose dependent manner (3). High doses are associated with severe side effects including diarrhea, vomiting, hair loss, etc. Therefore, there is an urgent need to develop progressively more effective tumor cell specific cytotoxic agents that are virtually non-toxic to the normal cells and tissues.

Photodynamic therapy (PDT) has become an important feature of biomedicine and is under intense investigation around the world, not only for the eradication of solid tumors but also for the extracorporeal purging of contaminated blood and its products. The clinical treatment regimen usually consists of systemic I.V. administration of Photofrin II, a porphyrin sensitizer currently undergoing clinical trials, followed by an equilibration period of 24-72 hours, during which time the photoactive compound is retained to a greater extent in tumor tissue than in some but not all, normal tissue according to majority of published reports. Illumination of the tumor - retained porphyrin with visible light leads to production of singlet oxygen, which is reported to be the toxic agent responsible for tumor regression and necrosis (4-6).

Many different dyes have been proposed and in particular, porphyrin and phthalocyanine derivatives are extremely popular materials. This subject has attracted the attention of synthetic chemists, who are actively engaged in designing new dyes which absorb at long wavelength because longer wavelength of light penetrates deeper into the target tissue (7-9). The main role of the photochemists has been to identify dyes which possess high triplet state quantum yields and which generate singlet oxygen in high yield upon irradiation in O_2-saturated solution (10). Dyes which meet this criteria are designated as potential PDT sensitizers, and dyes that do not produce significant yields of singlet oxygen receive no further attention because singlet oxygen is believed to be the sole

cytotoxic agent. However, this does not appear to be the case because: 1) the involvement of singlet oxygen in actual PDT processes is plausible, and in certain cases highly likely, but completely unproven; 2) there is not always a correlation between the observed cytotoxicity and yield of singlet oxygen. In addition to these unresolved fundamental issues, PDT suffers from a major limitation i.e it cannot be used for systemic therapy of malignant or viral diseases except in cases where target tumor is visible to the naked eye and accessible to the light via fiber optics and surgery. Thus, other possibilities such as involvement of photoproducts as at least additional cytotoxic agents must be considered.

Novel approach:

In consideration of these limitations and to enhance our understanding of the underlying mechanism(s), we have developed a novel approach termed *preactivation* in which an inactive photoactive compound is pre-illuminated under specific conditions. The singlet oxygen thus produced attacks the dye molecule itself and the process results in the formation of previously unknown photoproducts that exert their tumoricidal and/or virucidal effect without further requirement for light energy (11-13). Therefore, these photoproducts can be systemically administered as therapeutic agents for the treatment of malignancies (metastasized or not) and viral infections.

In previous preliminary studies we have shown that photoproducts in preactivated merocyanine 540 (pMC540) are effective against certain types of cancer cells, including breast cancer, and they are easily tolerated *in vivo* (14, 15). Some of the photoproducts of pMC540, namely meroxazole, merodantoin, and merocil, have been isolated and characterized. The percent yield of meroxazole, merocil, and merodantoin isolated from preactivated MC540 is 20.1%, 15.8% and 16.4%, respectively (16). The mechanism of action is not entirely clear but appears to involve induction of reactive oxygen species (17).

Purpose:

The purpose of this study has been to determine the efficacy of novel photoproducts in pMC540 against human breast cancer cells transplanted into nude mice. The MCF-7 cell line was originally isolated from a pleural metastasis of human breast adenocarcinoma and has been used as a model to study estrogen-dependent tumors. To determine the efficacy of novel photoproducts, MCF-7 cells grown as xenografts in immunosuppressed mice were used to determine the effect of pMC540 (with and without tamoxifen) and merodantoin on MCF-7 tumor growth *in vivo*. The results reported here demonstrate that even a brief period of treatment with novel photoproducts in pMC540 resulted in a dramatic suppression of the growth of human MCF-7 breast cancer xenografts without observable side effects as judged by the general well being of the treated animals.

Materials and Methods:

Chemicals: Time-release tamoxifen pellets (5 mg/pellet; 60-day release), and 17β-estradiol (1.7 mg/pellet, 60-day release) were obtained from Innovative Res. of America, Ohio, USA. Merocyanine 540 was purchased from Kodak-Eastman Fine Chemicals (Rochester, NY).

Preactivation of MC540: Merocyanine 540 (1 mg/mL) in 70% aqueous ethanol was preactivated by exposure to a bank of fluorescent lamps (GE cool white 40 W) for 18 hours (11-13). After preactivation, ethanol was removed by rotaevaporation, and the final concentration was adjusted to 50 mg/mL in 2.5% ethanol:PBS, pH 7.2. Merodantoin (an active photoproduct originally isolated and purified from pMC540) was synthesized in our laboratory and stock concentration was adjusted to 50mg/ml in dimethyl sulphoxide.

Synthesis of merodantoin: A solution of oxalyl chloride (0.040) in 15 ml of dry CH_2Cl_2 was added at -78°C with stirring to a solution of (0.035 mol) N, N' -di-n-butylurea. The

mixture was kept for 1 hour at -78° C and later allowed to warm up to room temperature. It was washed with water, dried over sodium sulphate and evaporated to yield a red-brown colored oil, which was washed through silica gel column with CH_2Cl_2/ethyl acetate (5:1). The eluate was further purified through a second silica gel column with CH2Cl2/n-hexane (3:1) to yield a yellow-red oil, which was identical with merodantoin obtained by photooxidation of MC540 according to FT-IR, NMR and MS data.

Cells and Animals: Human breast cancer cell lines MCF-7, T47D, BT474, MB231, MB436, and MB453 were purchased from American Type Culture Collection, and monolayer cultures were maintained in recommended growth medium and kept at 37°C in a humidified atmosphere of 5% CO_2 in air. BALB/c athymic nude-nu mice (6–8 weeks old, 18–22 g each) were purchased from Harlan Sprague-Dawley, Inc. and maintained in a germ-free environment. Sterile food and water were provided ad libitum.

In vitro screening: The effectiveness of pMC540 and merodantoin against a panel of breast cancer cell lines was determined. For this purpose breast cancer cells (5×10^4 cells/well) from different cell lines were plated in 96 well microtiter plates. After overnight incubation (22 to 24 hours) indicated concentrations of pMC50 or merodantoin were added. Cell viability was determined after 24, 48, 72 and 96 hours of continuous drug exposure by MTT assay (18). Results of three separate experiments each set up in quadruplicate are shown.

In Vivo Tumor Growth and Treatment: To determine the *in vivo* efficacy of novel photoproducts in pMC540 against human breast tumor, the MCF-7 breast adenocarcinoma cells were grown as solid tumor xenografts under the skin between the panniculus musculosus and fascia. This area is richly vascularized. Therefore, the blood supply and lymph drainage can be readily established. The free mobility of the skin allows an expansion of the skin over the growing tumors. MCF-7 tumor were grown in 17β-estradiol pellet–bearing mice (implanted by using a trocar needle 3 days before the injection of tumor cells). Solid tumors were then transplanted serially in estradiol pellet–bearing mice. For intratumor injections the implantation size of the tumor was 5x5mm and the treatment was initiated when the average size of the tumor reached approximately 7x6 mm. In experiments where intramuscular injections were employed as the mode of drug delivery, the implantation size of the tumor was 2x2 mm and the treatment of control group with vehicle only and experimental groups with pMC540, tamoxifen, and merodantoin were initiated 24 hours post tumor implantation. Tamoxifen pellets (5 mg/pellet) were implanted subcutaneously by using a sterile trocar needle. Preactivated MC540 and merodantoin were injected intramuscularly in the hind limbs alternately on alternate days for a period of 40 days.

In situ measurements of tumor xenografts were made using Vernier calipers, and L x W x H were recorded. At the end of the experiments, tumor xenografts were excised, and their wet weight and size were determined. The tumor volumes were measured by the water displacement method using a pycnometer. For the calculation of tumor area and tumor volume, the following formulas were used, respectively:

$$\text{Tumor area} = {}^{l}/_{2} \times {}^{w}/_{2} \times \pi; \quad (19)$$

$$\text{Tumor volume (V)} = Pw-(Pt-T)D \quad (20)$$

where l = tumor length, w=tumor weight; Pw=weight of pycnometer filled with water, Pt=weight of pycnometer filled with water plus tumor, T=tumor weight, and D=density coefficient of pure water.

Statistical Analysis:

Results are presented as the arithmetic mean x ± S.D. for each control and experimental group. Differences among the x of groups were determined using the Student two-tailed *t*-test, and p values < 0.05 were considered to be significant.

Results:

In a set of *in vitro* experiments, a number of breast cancer cell lines were treated with different doses of pMC540 and merodantoin. Results show (Table 1) that ductal carcinoma T47D and BT474 cells were susceptible to the cytotoxic action of pMC540. Similarly, MCF-7 adenocarcinoma cells were also susceptible (Figure 1). The pMC540 mediated cytotoxicity was time and dose dependent. However, the remaining cell lines MB231, MB436 and MB453 were not affected. These cell lines were also tested to determine the effect of merodantoin. Results show (Table 2) that all cell lines tested were susceptible to the action of merodantoin, producing approximately 90% cell kill at a dose of 48 μg/ml. The most sensitive cell lines in this group were MB436 followed by MCF-7 requiring 15 μg/ml and 25 μg/ml of drug to produce a 90% cell kill.

The dose response curves for pMC540 and merodantoin against MCF-7 breast cancer cell line are shown in Figures 1 and 2. For pMC540 the cytotoxic effect was dose and time dependent and irrespective of the dose the cytotoxic effect appears to proceed in a gradual manner. A maximum cell kill of 73% was observed at a dose of 120 μg/ml after 96 hours of incubation. However, for merodantoin (Figure 2) the effects were also dose dependent but prolonged incubation with this compound produced only marginal increase in cell kill indicating that the kinetics of cell kill are different for these two agents.

In the first set of *in vivo* experiments the effects of pMC540 administered via intratumor route was investigated as this method allows the direct exposure of the tumor to unmetabolized forms of the experimental compound. Athymic mice with MCF-7 cells growing as solid tumors received intratumor injections of pMC540 (250 mg/kg) on alternate days after tumor reached an average size of 7 x 6 mm. The size of the implanted tumors was 5 x 5 mm. Results show that the treated tumor growths were significantly ($p<0.001$) regressed (Table 3 and Figure 4). A palpable tumor in three animals out seven could not be detected. These results indicate that pMC540 treatment causes an antitumorogenic response to human breast cancer cells to an *in vivo* environment. These data provided the foundation for an *in vivo* model that can be used to study the antitumor effects of pMC540 as its active isolates against breast cancer as well as providing a surrogate human "target organ" in which to examine the antitumor effects of this class of novel compounds.

The next set of experiments were done to determine the efficacy of systemically administered photoproducts in pMC540 against breast tumors. For this purpose intramuscular route of injection was chosen because in pilot experiments this modality produced a more favorable response. The tumor growth curves for control and treated groups are shown in Figure 3. Data shows that active growth of the xenografted tumors continued for the duration of the experiments. In the presence of 1.7 mg estrogen pellets the doubling of tumor size in the control group was approximately 10 days which is in agreement with the previous reports (21).

Results show (Table 4, Figure 5) that treatment of athymic mice bearing human breast xenografts with pMC540 (250 mg/kg) resulted in a significant ($p=0.004$; $p=.0882$; $p=.0903$) reduction in tumor area, weight and tumor volume, respectively. In the group of animals receiving tamoxifen alone, the average area of the tumor was slightly larger as compared to the vehicle only control group and this difference was statistically

insignificant. This was probably due to significantly higher affinity of estradiol for estrogen receptors as compared to tamoxifen as well as higher than previously used concentrations (0.1 to 1.0 mg) of estradiol (21, 22). This virtual inactivity of tamoxifen is probably due to the low dose of tamoxifen used. At higher doses tamoxifen has been shown to be an effective cytostatic agent against breast cancer. However, in the group of animals receiving a combination of tamoxifen and pMC540, the reduction in tumor area, weight and volume was highly significant ($p=0.0002$;$p=0.029$; $p=0.0069$) respectively, even though the dose of pMC540 was reduced from 250 mg/kg to 100 mg/kg. These results suggest that a combined use of these two compounds may be more effective. When this treatment group was compared with the tamoxifen alone group, the p values were even lower, indicating an extremely significant difference between these two groups. Since the plasma half-life of pMC540 is approximately 6 hours (14), the efficacy of this compound may be improved by manipulating the dose, dosing regimen, and/or a slow-release drug-carrier system.

Treatment of a fifth group of animals bearing human breast tumor was carried out using merodantoin, a chemically synthesized photoproduct present in pMC540. Results show (Table 4, Figure 5 and 6) that in two of the six animals, the implanted tumor could not be detected; in the remaining four animals, the implanted tumor did not grow beyond the implantation size. These results suggest that a purified form of photoproduct is significantly more effective than the mixture of photoproducts. The mechanism of action of photoproducts is not entirely clear but appears to involve the intracellular induction of reactive oxygen species leading to alterations in calcium homeostasis, DNA damage and apoptosis (unpublished data). Data presented lends further support to our contention that singlet oxygen is not the only toxic agent produced during photosensitization and does involve previously unknown photoproducts. Once produced these products are long lived and they function independent of light. Determination of the efficacy of merodantoin against established human breast tumor is under way.

Both pMC540 and merodantoin were easily tolerated without observable difference in the well-being of the treated groups. **The goal of this research is to determine the efficacy of a novel class of compounds generated via the process of preactivation, against human breast tumor *in vivo* models.** Data obtained thus far is most encouraging as only a brief treatment with novel compounds produced a dramatic suppression of tumor growth without observable side effects. Studies are underway to determine the effect of pMC540 and merodantoin in the presence and absence of tamoxifen on the established breast tumors.

Conclusions:

The completed research thus far indicates that preactivated merocyanine 540 by itself and in combination with anti-hormonal drug tamoxifen is effective in suppressing the growth of the breast tumors. Merodantoin, a chemically synthesized active isolate from pMC540 was most effective as only a brief treatment with this compound suppressed the breast tumor growth by 98%. These results suggest that photoproducts generated via the process of preactivation may be clinically useful.

Future work:

The promising results obtained thus far has raised more questions that need to be addressed in order to gain important information for the potential clinical utility of these compounds. For example, are these compounds effective against tamoxifen resistant tumors which is currently a major problem in the treatment of breast cancer ? It is not known whether the regrowth of breast tumor occurs or not once the therapy is stopped. If the regrowth of tumor occurs, is it controllable by a re-initiation of the therapy ? Is a

combination of intratumor and intramuscular injection of the drug more efficacious ? Does the therapy with these novel compounds prevent metastases of breast tumor ? Are these compounds effective against metastasized breast tumor ?

Problems:

Our proposed plan to convert chemically synthesized photoproducts namely merocil and merodantoin into time released drug pellets was not successful. This was due to the fact that these products turned out to be viscous oily substances, whereas dry powder form is required to pelletize drug products. This was disappointing because it was envisioned that continuous release of the drug should produce more efficacious results as well as reduce the handling of animals.

The chemical synthesis of merodantoin has progressed smoothly however, the synthesis of the second photoproduct (Merocil) has turned out to be more difficult and as a result the availability of sufficient quantities has been delayed until February -March 1994. Thus if unforeseen problems are not encountered, the *in vivo* efficacy of this compound is expected to be evaluated by August, 1994.

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Table 1 Effect of preactivated MC540 on breast cancer cell lines

Cell Type	pMC540 (μg/ml)	Percentage Cell Kill (mean ± S.D.)			
		24 Hours	48 Hours	72 Hours	96 Hours
T47D, ductal	40	3	8	12	62.3 ± 9.7
carcinoma	80	16	5	15	93.0 ± 4.0
	120	22	11	33	94.0 ± 4.4
BT474, ductal	40	0	1	7	17.0 ± 5.6
carcinoma	80	13	1	6	66 ± 6.2
	120	14	11	15	80.7 ± 1.0
MB231, breast	40	0 ± 0	1.0 ± 1.0	5.0 ± 1.0	1.0 ± 2.3
adenocarcinoma	80	0 ± 0	1.7 ± 2.1	8.0 ± 1.7	2.4 ± 4.8
	120	0.33 ± 0.58	7.0 ± 6.1	8.0 ± 0	4.4 ± 5.9
MB436, breast	40	0 ± 0	4.7 ± 4.5	5.0 ± 8.7	0 ± 0
adenocarcinoma	80	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	120	0 ± 0	0 ± 0	0 ± 0	0 ± 0
MB453, Breast	40	3.0 ± 3.0	3.0 ± 3.6	13.0 ± 3.6	0.33 ± 0.58
carcinoma	80	3.67 ± 1.2	2.0 ± 1.7	13.3 ± 2.9	0.67 ± 0.58
	120	3.3 ± 1.2	9.7 ± 1.5	15.3 ± 2.1	1.7 ± 1.5

Table 2 Effect of merodantoin on breast cancer cell lines

Cell Type	Merodantoin (μg/ml)	Percentage Cell Kill (Mean ± S.D.)			
		24 Hours	48 Hours	72 Hours	96 Hours
T47D, ductal carcinoma	6				32.7 ± 4.0
	12	43	50	70	52.3 ± 4.0
	24	35	48	71	69.0 ± 13.0
	36				79.0 ± 11.4
	48				90.0 ± 7.8
	60				92.0 ± 4.4
BT474, ductal carcinoma	6				28.3 ± 1.0
	12	16	17	49	43.7 ± 4.5
	24	25	44	67	64.7 ± 3.5
	36				80.0 ± 2.6
	48				91.7 ± 3.5
	60				91.7 ± 3.2
MB231, breast adenocarcinoma	6	18.7 ± 8.1	42.0 ± 6.2	37.7 ± 2.1	7 ± 2.6
	12	36.0 ± 7.5	71.3 ± 0.58	68.7 ± 2.1	24.3 ± 2.1
	24	52.0 ± 9.5	81.0 ± 6.9	73.7 ± 0.58	62.0 ± 2.6
	36	52.3 ± 13.6	95.3 ± 2.1	90.3 ± 2.5	87.0 ± 2.6
	48	83.3 ± 12.5	94.3 ± 1.5	93.7 ± 1.5	93.0 ± 1.0
	60	68.3 ± 6.5	95.0 ± 4.0	94.0 ± 1.0	97.3 ± 1.5
MB436, breast adenocarcinoma	1	21.3 ± 15	17.7 ± 6.8	15.7 ± 7.4	26.7 ± 13
	5	40.7 ± 13	41.7 ± 11	50 ± 12	54.7 ± 10
	10	43 ± 7.0	60 ± 6.2	63.7 ± 11	74 ± 6.0
	15	50 ± 12	64 ± 8.7	75.7 ± 6.7	89 ± 6.2
	25	71 ± 15	82.7 ± 7.4	91.3 ± 8.1	95.7 ± 4.0
	35	74.7 ± 18	87.7 ± 5.5	89.3 ± 11	94.7 ± 4.7
MB453, breast carcinoma	6	12.0 ± 2.6	8.0 ± 3.0	10.7 ± 7.1	1.7 ± 2.9
	12	42.3 ± 2.1	59.0 ± 1.7	57.0 ± 3.6	5.0 ± 3.0
	24	69.7 ± 7.0	66.3 ± 2.1	72.3 ± 1.5	51.7 ± 5.1
	36	68.0 ± 2.0	97.0 ± 1.0	96.0 ± 1.7	96.0 ± 1.0
	48	90.7 ± 1.5	97.0 ± 1.0	96.7 ± 0.6	96.7 ± 0.6
	60	90.0 ± 3.0	96.7 ± 1.5	96.3 ± 1.2	96.7 ± 1.5

Table 3. Effect of intratumor injections of pMC540 on MCF-7 breast tumor transplanted into athymic mice.

Treatment Group	Dose	Tumor Area, cm^2 (on last day)	Tumor Weight, g (mean±SD)	Tumor Volume, cm^3 (mean±SD)
Control (n=7)	—	10.532±5.244	0.719±0.4326	0.4981±0.4399
pMC540 (n=7)*	250 mg/kg	3.7116±0.6948 p=.0026	0.2645±0.1116 p=.0121	0.1193±0.0615 p=.0302

Route of drug administration: intratumor, 20 doses on alternate days.
Size of tumor implanted: 5 x 5 mm.
*Three animals did not show any palpable tumors at the end of the treatment.

Table 4. Effect of pMC540, Tamoxifen, Tamoxifen + pMC540, and Merodantoin on the Growth of MCF-7 Breast Tumor.

Treatment group	Dose	Tumor area (cm^2)	Tumor weight (g)	Tumor volume (cm^3)	%TGI
Control* (n=10)	Vehicle only	10.780±1.44	0.797±0.141	0.800±0.141	
pMC540 (n=10)	250 mg/kg	5.030±1.02 (p=.0044)**	0.455±0.123 (p=.0882)	0.471±0.118 (p=.0903)	42.91
Tamoxifen (n=10)	5-mg pellet/ mouse	11.090±1.700 (p=.8909)	1.059±0.177 (p=.2604)	1.058±0.172 (p=.2609)	
Tamoxifen plus pMC540 (n=10)	5-mg pellet + 100 mg/kg	3.310±0.710 (p=.0002)	0.263±0.071 (p=.029)	0.311±0.077 (p=.0069)	67.0
Merodantoin (n=6)	75 mg/kg	0.211±0.104 (p=.0001)	0.011±0.004 (p=.0009)	ND	98.62

*Implantation size approximately 0.2 cm^2. Treatments with pMC540 and merodantoin were given on alternate days via intramuscular injections for 40 days. Except for tamoxifen, all treatments caused a significant suppression of MCF-7 tumor growth.

%TGI indicates percent tumor growth inhibition, calculated by using the formula $\%TGI=100(1-W_t/W_c)$, where W_t and W_c are the mean weights of the treated and control tumors, respectively.

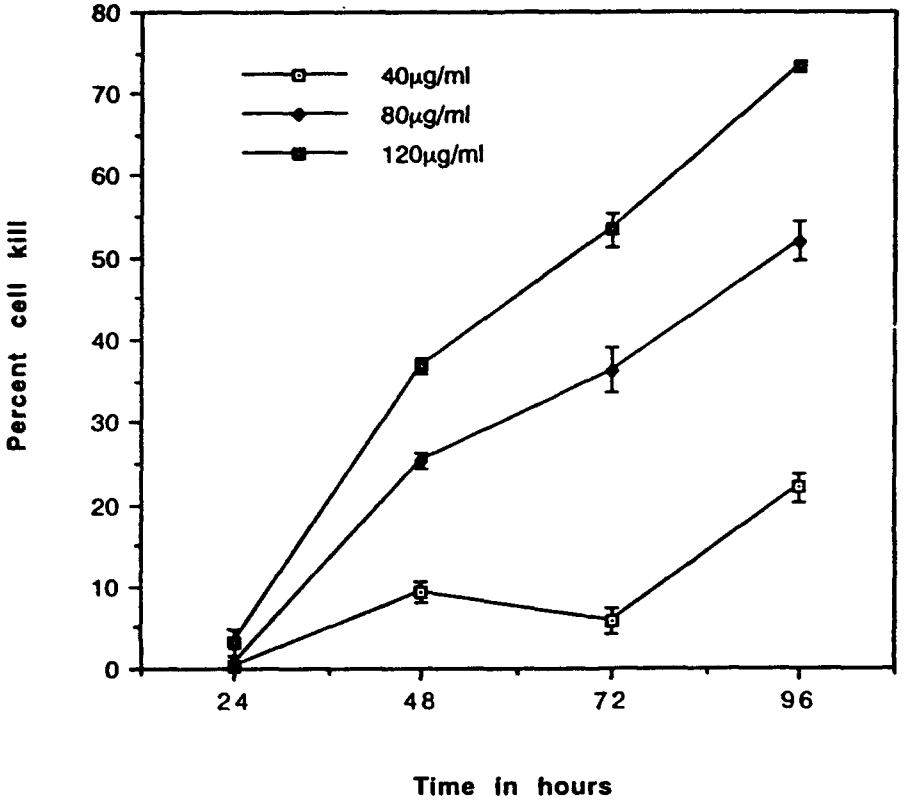

Fig. 1. Dose-effect curves of pMC540 against MCF-7 cells. Cells (5 x 10^4 cells/ml) were treated with indicated doses of pMC540. After incubation at 37 ^{o} C for 24, 48, 72 or 96 hours, the cell viability was determined by MTT assay. Results (mean ± s.e.m) of three separate experiments performed in quadruplicate are shown.

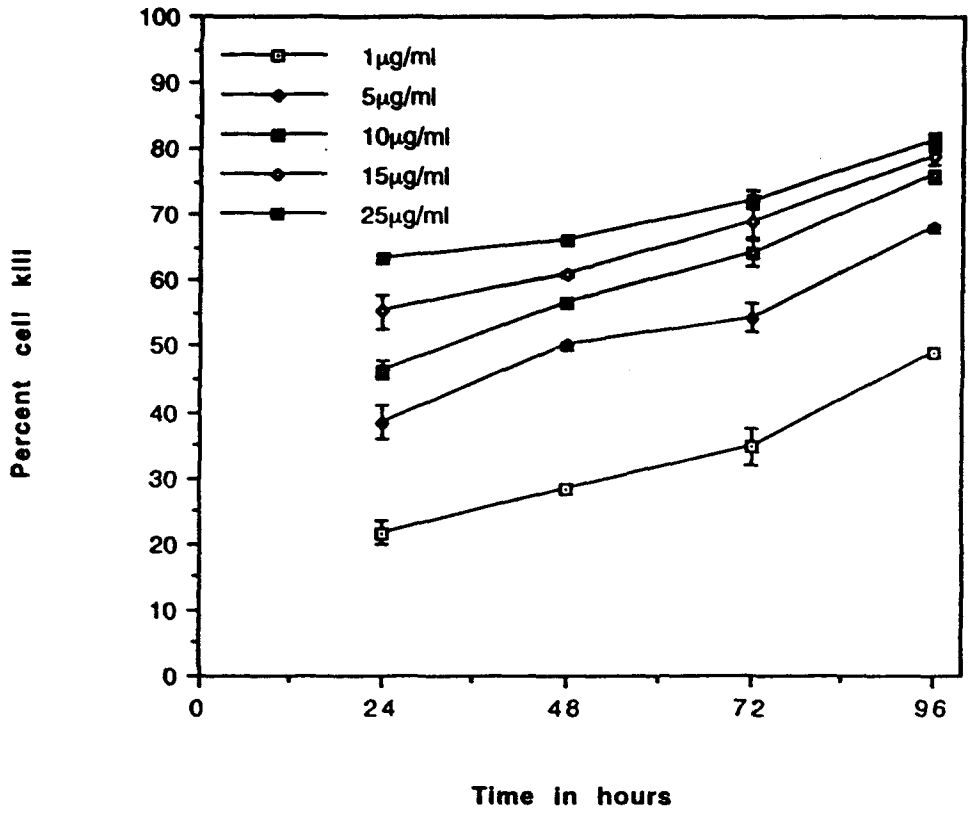

Fig. 2. Dose-effect curves of merodantoin against MCF-7 cells. Cells (5 x 10^4 cells/ml) were treated with indicated doses of merodantoin. After incubation at 37 ^{o} C for 24, 48, 72 or 96 hours, the cell viability was determined by MTT assay. Results (mean ± s.e.m) of three separate experiments performed in quadruplicate are shown.

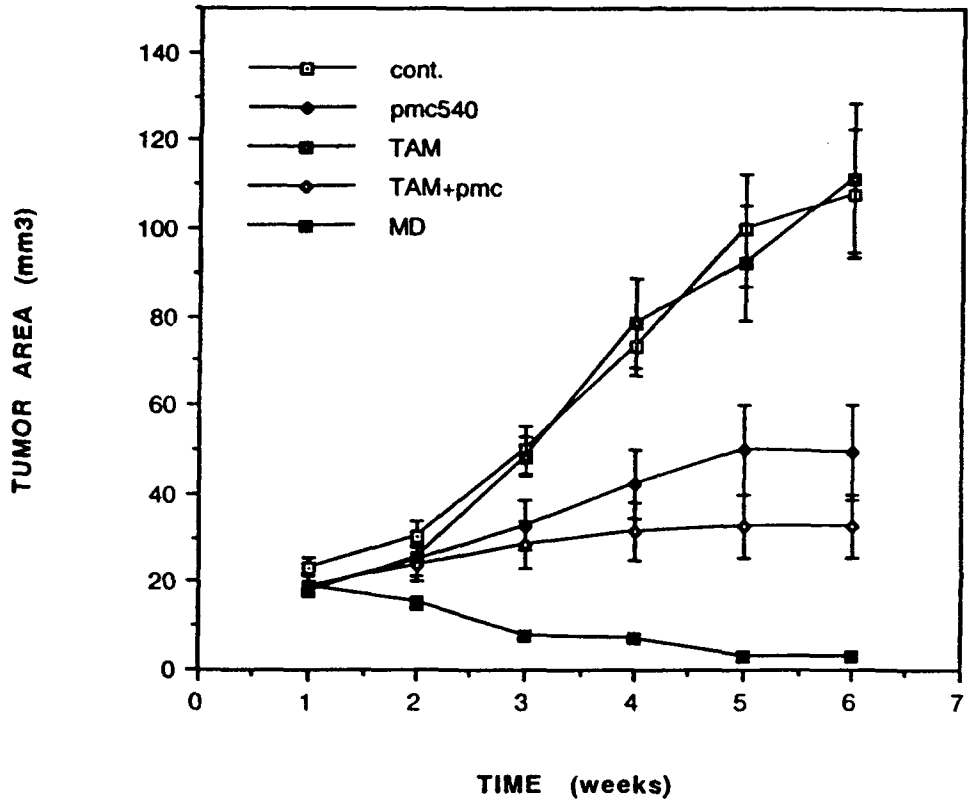

Fig. 3. Tumor growth (mean + s.e.m.) curves for MCF-7 xenografts in the presence of continuous estrogen and treated with pMC540 (250 mg/kg), tamoxifen (5 mg pellet), tamoxifen plus pMC540 (5 mg pellet +100 mg/kg) and merodantoin (75 mg/kg). Alternate day treatment of mice was initiated a day after tumor implantation. There were 10 mice per group.

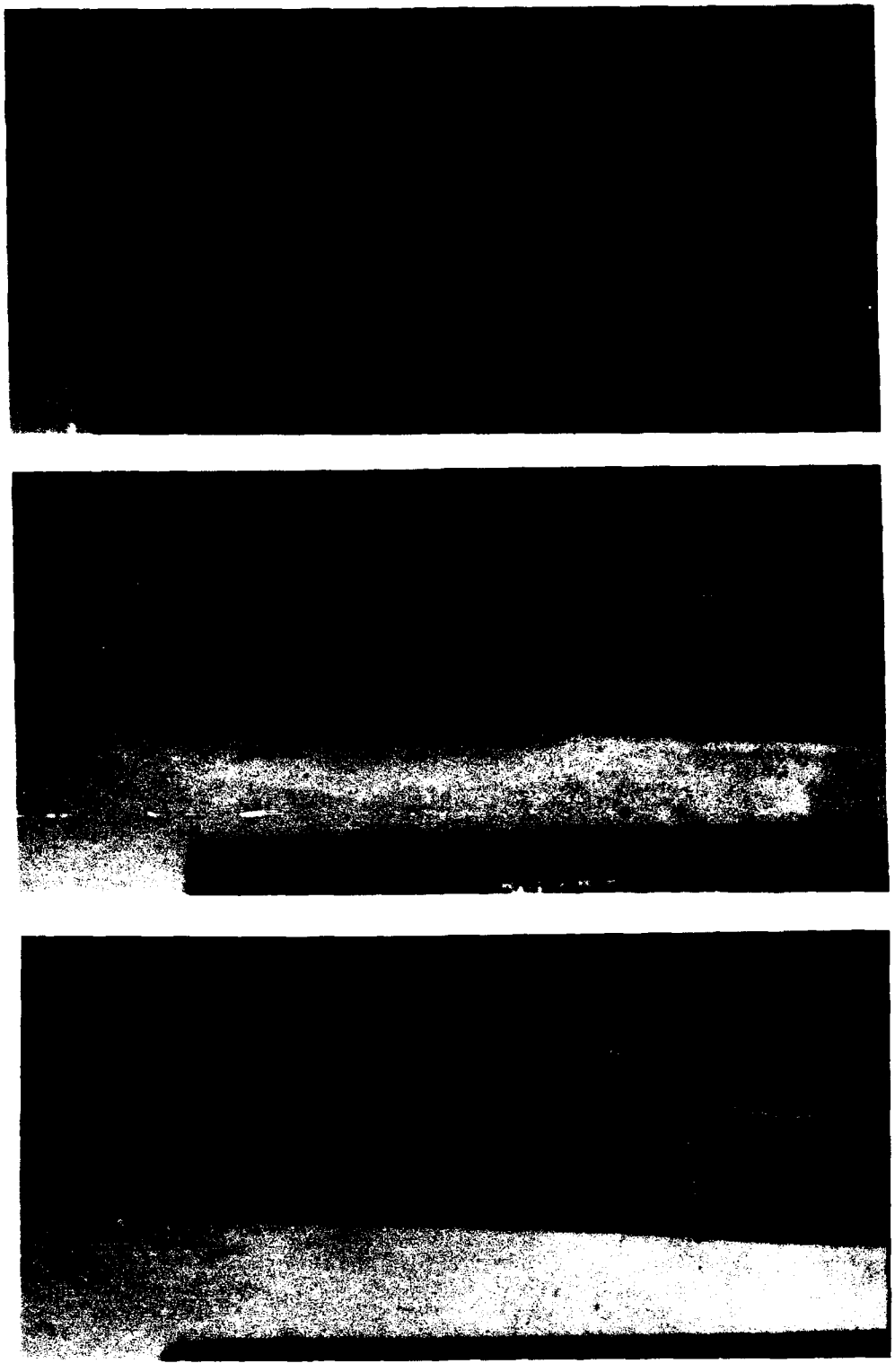

Figure 4. Vehicle control (top) treated with intratumor injections of pMC540 (250 mg/kg), during treatment (middle), at the end of the 20 dose treatment (bottom).

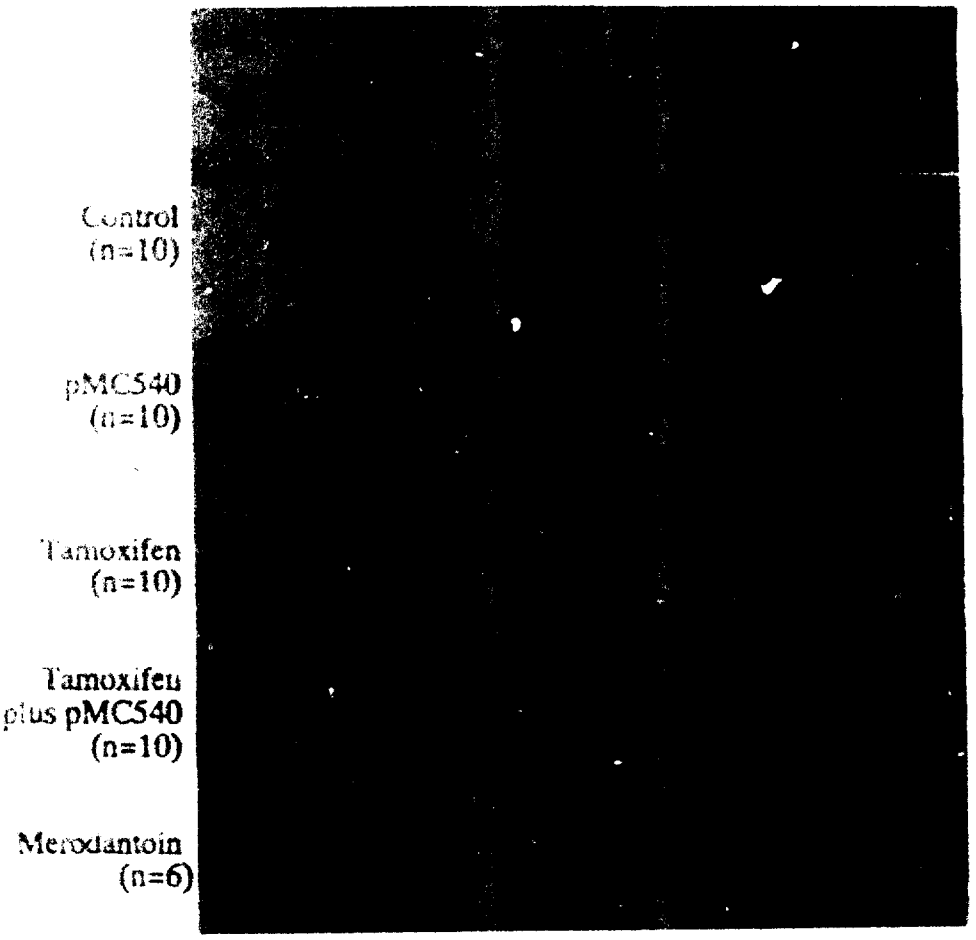

Figure 5. Photograph depicting the excised tumors from control and treatment groups. Each row in the group shows (from left to right) three tumors representing the largest, median, and smallest tumors within the group. This photograph corresponds to Table 2.

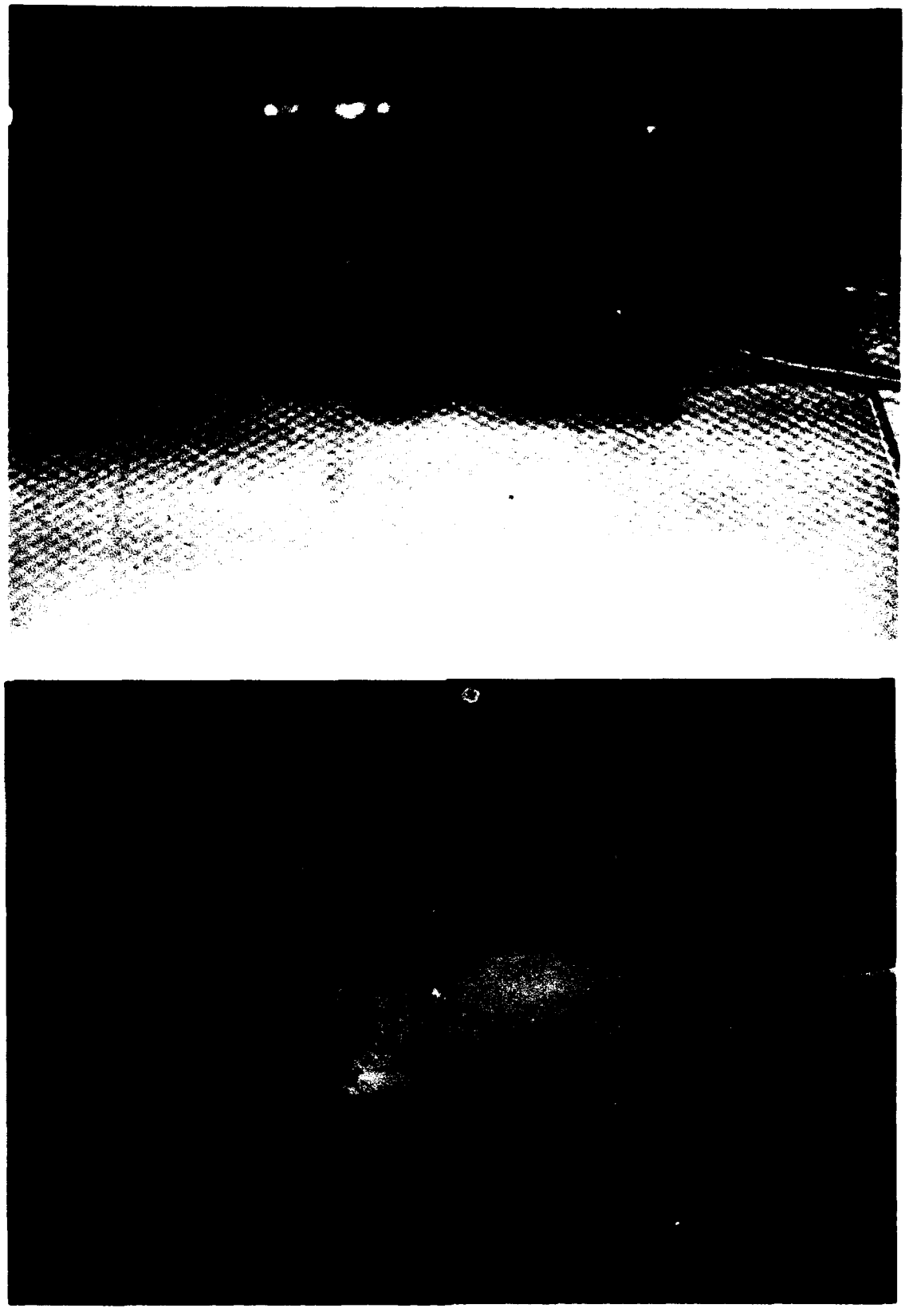

Figure 6. Vehicle control (top), treated with 20 doses of mercodantoin (75 mg/kg) on alternate days (bottom).